The University of Chicago
FOUNDED BY JOHN D. ROCKEFELLER

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SPOROGENESIS IN PALLAVICINIA

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF SCIENCE, IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

BY

ANDREW C. MOORE

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SPOROGENESIS IN PALLAVICINIA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXV.

ANDREW C. MOORE.

(WITH PLATES III AND IV)

THE presence of a quadripolar spindle in the division of the spore mother cell of *Pallavicinia decipiens* was announced by FARMER in 1893, and in 1894 he published his detailed studies upon the same plant. The most remarkable feature of these papers is the significance which the author attributes to a quadripolar spindle as a means for the simultaneous distribution of the chromatin to the four daughter cells which become the spores.

According to FARMER'S account (**5, 6**), the structure in question is developed quite early, before any evidence of approaching division is visible in the nucleus. Later the nucleus becomes lobed, and finally four chromosomes make their appearance. The number is increased by division to eight, which point off in pairs to the four lobes of the spore mother cell. "A further doubling of the chromosomes occurs, so that four of these bodies . . . . go to form the nucleus in each spore. The whole process is very much crowded up, the four-rayed spindle persisting to the end; and even after the exodus of the chromosomes, traces of it can still be seen converging to the original center."

The presence of a quadripolar spindle is of itself not surprising, since tripolar, quadripolar, and multipolar spindles have been frequently described by various authors; but in every case these structures represent early stages in the development of the achromatic figure and later become normal bipolar spindles. The peculiar

interest attaching to the structure described by FARMER is the reported distribution of the chromatin simultaneously to the four daughter nuclei. If his observations and his interpretation of the spindle are correct, Pallavicinia occupies a unique position among plants and animals.

FARMER (7) sought through a study of other liverworts to throw further light on this subject. He found the quadripolar spindle present in *Aneura pinguis*, *A. multifida*, *Scapania undulata*, Fossombronia, and in other types of the Jungermanniales, but in no case did he find it persisting and functioning, as in Pallavicinia, in the simultaneous distribution of the chromatin. In these forms, according to his interpretation, the ends of the quadripolar spindle fuse in pairs and the distribution of chromatin takes place in the usual manner through two successive mitoses. While not directly confirming his results on Pallavicinia, FARMER thinks the conditions found in these plants strengthen his position. He regards them as representing transitional stages between the normal type of division and the very unusual type which he reports in Pallavicinia. CAMPBELL (1) and other authors generally have accepted FARMER'S account.

DAVIS (4) from an investigation of Pellia was led to question FARMER'S conclusions. He regards the quadripolar spindle as a condition of prophase, and believes that it is always followed by two successive mitoses after the usual manner in the spore mother cell, each with a normal bipolar spindle. FARMER (8) is not willing to exclude the four-rayed figure from the spindle apparatus or to employ the term spindle in the restricted sense of DAVIS; but the main features of the discussion are not the questions as to when the achromatic structure becomes a spindle and as to the limitation of this term as a matter of usage—points upon which authors may readily disagree. The fundamental differences between the views of DAVIS and FARMER lie in the history of the quadripolar spindle, and the method by which the chromatin in the spore mother cell is distributed to the four spores. FARMER positively asserts that the quadripolar spindle retains its form and that the chromatin is distributed simultaneously to the four daughter nuclei. DAVIS believes that the quadripolar spindle is a condition of prophase which is followed by two successive

mitoses, each with bipolar spindles, by which the chromatin is distributed in the usual way within the spore mother cell. Apart from the rapidity of the two mitoses and the prominence of a four-rayed achromatic figure in the prophase of the first, the latter author holds that there is no essential difference between the processes of sporogenesis in Pallavicinia and in other liverworts and higher plants. DAVIS (4a) further maintains these opinions in his recent review of the events of nuclear division within the spore mother cell.

In view of the unusual character of FARMER'S results and of the fact that doubt has been expressed as to the accuracy of his observations and their interpretation, I have undertaken an investigation of *Pallavicinia Lyellii*, believing that evidence obtained from the study of another species of the same genus would help in clearing up the disputed points. Some of my results (15) have already been published, and they do not confirm FARMER in his main contention, namely, the simultaneous distribution of the chromatin.

Pallavicinia Lyellii is a cosmopolitan species which I have found growing abundantly near Columbia, S. C., and in the vicinity of Woods Holl, Mass. The young sporophytes make their appearance in the early fall and mature about the first of April. The material was fixed in chromo-acetic acid and stained with saffranin and gentian violet alone, or in the triple combination of saffranin, gentian violet, and orange G. Iron-alum haematoxylin was also used after the method of Haidenhain. Upon the whole the last-named stain has given the best results. The fibrillar structures are not so well brought out by it as by the gentian violet, but the chromosomes are much more clearly differentiated.

The spherical resting nucleus occupies a central position in the distinctly four lobed spore mother cell. It enlarges considerably preparatory to division and becomes somewhat angular, extending into the lobes of the spore mother cell. At the period of synapsis the nucleolus is conspicuous for its size and prominence (*fig. 1*), as is also the confused tangle of chromatic threads. The spore mother cell is not so deeply lobed as FARMER (8) figures for *Pallavicinia decipiens*, and DAVIS (4) and CHAMBERLAIN (2) for Pellia.

FARMER did not observe the spirem of *Pallavicinia decipiens*. In *P. Lyellii* it is exceedingly well-developed, and immediately after

synapsis is observed as a very definite linin thread in which deeply staining chromatic droplets (*fig. 2*) occur at intervals. This spirem is loosely wound in many convolutions through the nuclear area and shows no signs of fine anastomosing filaments uniting its parts. The nucleolus is not so conspicuous as in the preceding stages of synapsis.

The spirem thread shortens and thickens and the chromatin granules become larger and less numerous (*fig. 3*). This process continues until the length of the whole thread is not more than that of the circumference of the nucleus, though it does not always occupy a peripheral position. During the latter part of this shortening process, there is a crumpling of the thread and a crowding together of its chromatin granules. This condition is of comparatively short duration and it is at this point that I observed the first evidence of a double thread (*figs. 4* and *5*).

The thread presently segments into eight chromosomes which lie scattered about in the nucleus in the form of a ring. These eight chromosomes are irregular in shape and frequently show with great clearness that they are not homogeneous masses, but made up of individual parts. I was at first in much doubt as to the number of parts, but subsequent study has convinced me that there are four, and that we are dealing here with tetrads. FARMER (7) shows very clearly by his figures of Fossombronia that he saw a similar arrangement. He says "sometimes four such aggregations could be seen in each chromosome, but the number was not sufficiently constant to afford very secure ground for theorizing." However, he expresses the opinion that we have here a double longitudinal split, in which the chromosomes are already prepared for the two succeeding divisions.

The tetrads are clearly shown in *fig. 8*. The appearance of several of the eight chromatic masses suggests very strongly that they are made up of four parts, while the evidence presented by the one in the center is conclusive. Here there is present the ring form, and the four elements of the tetrad are quite distinct. Several of the forms assumed by the tetrads are illustrated in *fig. 10*, viz., crosses, Ys, Ts, and rings. The fourfold nature of the group is most clear in the ring form. In *fig. 10 a* the four daughter chromosomes of the

tetrad are shown entirely separated from one another. *Fig. 11* illustrates another case where the daughter chromosomes of the tetrad are separated. In this figure the two groups are drawn in correct relative positions, the one showing an almost homogenous mass, the other four daughter chromosomes. The fourfold character of the chromatic masses is most evident immediately after the segmentation of the chromosomes. Very soon they become more compact, and while they continue to show irregularities in outline, up to the metaphase of mitosis, they are not so evidently composed of four elements. *Fig. 9* presents a stage somewhat later than *fig. 8*.

I have not been able to determine the origin of the tetrads with any certainty. *Fig. 6* would seem to indicate that the elements of the tetrads are formed previous to the segmentation of the spirem and that these in some way become properly grouped. The large number of chromatic elements, together with the differences in their size and shape in this figure, are no doubt to be correlated with the different degrees to which the aggregations have progressed in the formation of the tetrads. In *fig. 7* the number of masses has been reduced, their size is approximately uniform, and the time has almost arrived for the segmentation of the chromosomes. Until the origin of these tetrads can be made out definitely, it would be useless to theorize concerning them.

While the foregoing changes take place within the nucleus, the outer form of that body is altered. It becomes strongly lobed, often assuming a tetrahedral form, one angle projecting into each lobe of the spore mother cell. FARMER (7) describes a similar form in several of the Jungermanniales studied by him, and attributes it to a pull by the four centrosomes which he finds in the four lobes of the mother cell. In describing the process for Fossombronia he says "the nuclear wall is not broken, although it becomes greatly pulled out beneath each centrosphere, and thus the quadripolar spindle is so far a nuclear distortion."

While the tetrahedral form is perhaps the most usual at this stage, it is by no means the only one. Frequently there are more than four projections. Such a condition as is illustrated in *fig. 12* would require the assumption of more than four centrosomes. In many cases the lobes of the nucleus are rounded and do not indicate

that they are caused by a pull upon the nuclear membrane (*fig. 3*). Besides, the membrane in sections can be seen to be wavy, showing that it is not under tension from a dynamic center. The lobing occurs long before fibrillar elements are visible, and if the assumption that fibers are the expression of lines of force be true, then such lines of force do not exist at this time, and hence the irregularities in the shape of the nucleus cannot be attributed to a pull by them. It would seem much more probable that they are due to an amoeboid movement of the nucleus. It is well known that the nucleus of certain cells possesses this power, and observers have noted the phenomenon in living cells. It has also been noted that there is in a measure a correspondence between the shape of the nucleus and that of the cell to which it belongs. When the cell is much attenuated the nucleus is greatly elongated. In *fig. 28* is shown a resting nucleus from an elater of Pallavicinia. Miss MERRIMAN (**14**) discusses this question in relation to the differentiation of tissues from the meristem in the root tips of Allium and attributes to the nucleus the power of amoeboid motion. KORSCHELT (**12**) describes in the egg of the water beetle Dytiscus a nucleus with pseudopodia-like processes extending out into a mass of granular food particles.

As previously stated, the resting nucleus of the already deeply lobed spore mother cell is spherical. In preparation for division the great changes which take place in its size and in the character of its contents must be connected with great metabolic changes going on within it. The materials necessary for the supply of this demand must come from the cytoplasm, which in this case consists of four masses occupying the four lobes of the spore mother cell, and the reaching out of the nucleus for food might tend to produce a tetrahedral form.

According to FARMER the quadripolar spindle was the first evidence of approaching division. He says the first evidence noted in the nucleus itself was the collection of four chromatic droplets in the center at a time subsequent to the appearance of the four-rayed structure. In *Pallavicinia Lyellii*, as has already been pointed out, the changes which take place in the nucleus itself indicate approaching division before any structure makes its appearance which could be interpreted as a quadripolar spindle.

FARMER does not discuss the origin of the achromatic spindle, evidently regarding that as a matter of minor importance as compared with its later behavior in his account of the simultaneous distribution of the chromatin. The study of the origin and development of the achromatic structure of *Pallavicinia Lyellii* is attended with considerable difficulties, owing to the large number of chloroplasts in the cell. However, it seems to conform in general to the type described by DAVIS (4) for the corresponding phase of Pellia. He finds that kinoplasmic caps form over the lobes of the nucleus and extend down over it, finally forming fibrillae which enter the nuclear area. In my preliminary note (15) I described a similar process for Pallavicinia. I found aggregations of kinoplasm at the angles of the nucleus, and out of this material fibers are formed, which extend down over the protruding portion of the nucleus. FARMER (8) has recognized in one of my figures representing this stage the same structure as his quadripolar spindle.

In *P. Lyellii* this structure is never so prominent as that described by FARMER, but his figures do not distinguish clearly the spindle fibers from the nucleus. My preparations show a decided lobing of the nucleus, but with very slight indications of differentiated fibrillar protoplasm over the lobes. I find no astral rays and no evidence whatever of the existence of centrospheres or centrosomes. DAVIS (4), CHAMBERLAIN (2), and GRÉGOIRE and WYGAERTS (9) find asters and kinoplasmic caps well developed in other periods of ontogeny, but do not find them so prominent, if at all, in the spore mother cell. FARMER indeed does not mention the presence of asters in Pallavicinia, nor does he figure them. DAVIS (3) in his investigation of Anthoceros was the first to question the presence of centrosomes in the spore mother cell of liverworts. My studies lead me to hold similar doubts and to believe with him that the spindle fibers in the spore mother cells of liverworts develop independently of centrosomes, so that multipolar stages in spindle formation may be expected, as OSTERHOUT, MOTTIER, and JUEL established in 1897 in the pteridophytes and spermatophytes.

CHAMBERLAIN (2), who studied the germinating spore of Pellia with special reference to the centrosome problem, describes a peculiar structure in the form of a vesicle fitting over the end of the nucleus

and in this he is confirmed by GRÉGOIRE and WYGAERTS (9). This vesicle, which he interprets as a *Hautschicht*, resolves itself into fibers and furnishes at least a part of the material for the spindle. I do not find such a vesicle separate and distinct from the nuclear membrane, but I find strong evidence that the nuclear membrane itself becomes resolved into fibers. This view is quite compatible with the generally accepted theory of the nature of a plasma membrane, and the evidence is presented by such appearances as are shown in *figs. 12–14*. In *fig. 12* we have a nucleus which in one plane shows a number of prominent lobes. A few fibers are visible over one lobe, and at several other places the nuclear cavity is apparently bounded by a weft of fibers. These are either derived from a layer of kinoplasm which closely invests the nucleus or from the nuclear membrane itself. The fact that the nuclear membrane disappears as these fibers come into view would lend force to the latter supposition. In *fig. 13* fibers are shown over one lobe of a nucleus which is very much elongated, and in *fig. 14* they may be seen at both ends of a similarly elongated nucleus. In the latter case the nuclear membrane persists in several places, seeming to merge gradually into the fibrillar condition. The fibers appear to conform to the irregularities of the surface, giving strong indications that they are derived from the nuclear membrane.

HARPER (10) has shown a close relation between membranes and fibers in Erysiphe, where, in free spore formation in the ascus, the fibers which mark out the boundary of the future spore fuse side by side to form a plasma membrane. The nuclear membrane is generally believed to be of kinoplasmic origin, and so are the fibers of the achromatic spindle. Evidently then, the transition from the one to the other may be easily accomplished.

Soon after the appearance of the first fibers, the number is greatly increased, but I have not been able to determine the origin of the remainder. The completed spindle is bipolar, and may be pointed (*fig. 15*) or blunt (*fig. 16*). The ends may terminate near or at a distance from the cell wall. It happens frequently that one end extends into a lobe of the spore mother cell, and the other abuts on the infolded wall between the two adjacent lobes which stand opposed to it, thus producing a very much flattened pole or even a forked one (*fig. 16*).

At the completion of the achromatic spindle, the chromosomes are found grouped in a ring at the equatorial region of the structure. *Figs. 8* and *9* show the arrangement of the chromosomes at this stage. *Fig. 15* gives a side view, slightly oblique, of the chromosomes at metaphase of mitosis. Five chromosomes are in view and the other three are hidden or have been removed by the razor in making the section.

I have not been able to make out satisfactorily the details of the separation of the daughter chromosomes. The distribution is effected very quickly, for great numbers of nuclei in metaphase have been observed and a great many in telophase, but very few in anaphase. Little indication is given as to the exact manner in which the separation takes place. A few instances of chromosomes as they are pulled apart are shown in *fig. 17*. The appearance of the chromosomes indicates beyond doubt that they are plastic bodies subjected to a pull, and that they are being halved; but what real relation this distribution bears to the original tetrads is left in doubt. In *fig. 18* we have shown anaphase in which the chromosomes are somewhat scattered upon a very broad spindle. There are five near each pole and one almost half way between. It is evident that the remaining chromosomes are upon another section.

During telophase the chromosomes are found arranged in compact rings at the two poles. When one end of a spindle abuts on a dividing wall between two lobes, the ring at that end sometimes lies very close to this wall, partially surrounding it (*fig. 19*).

There is no resting stage between the first and second mitoses. The chromatic elements of the nucleus do not resolve themselves into a reticulum and the chromosomes do not lose their individuality. The rings of chromosomes which have been formed at the telophase of the first division merely alter their positions, so that their planes lie at right angles to one another. It is evident from *fig. 20* that the chromosomes come in contact and form a thick spirem, but do not lose their identity. This is the nearest approach to a resting stage I have been able to find, and I believe it is unusual for the reconstruction of the nucleus to proceed even this far. No nuclear membrane is formed at the end of the first mitosis and no cell plate is laid down. In a few instances granules were seen across the equatorial portion

of the spindle, but the process of forming a wall seems to go no further; indeed, it very seldom proceeds to this point.

That the second mitosis succeeds the first very closely is attested by the fact that examples of both divisions are frequently found in the same capsule. The spore mother cells of a given capsule are in division at the same time, though not exactly in the same phase of mitosis. Occasionally cells are found which lag considerably behind or precede the majority in division. Such cases are of great value in determining stages with certainty.

The spindles for the second mitosis make their appearance very suddenly, and I have not been able to determine their origin. They are quite strongly developed, and as a rule are longer and narrower than the spindles of the first mitosis. The passage from the metaphase to the telophase is almost as rapid as in the first division, and no additional evidence is afforded as to the manner in which the chromosomes separate. *Fig. 21* illustrates metaphase of the two spindles, showing a polar and a side view. In this example the poles of the spindles are sharply pointed. In *fig. 22*, which represents an anaphase, the poles are blunt. The chromosomes pass rapidly to the poles and are grouped at the two ends in rings (*figs. 23* and *24*). At this stage the fibers are very prominent in transverse sections of the spindle (*fig. 24*).

Soon after the chromosomes have passed to the poles, granules make their appearance upon the equatorial region of the spindle (*fig. 24*). These become divided and a cell plate is laid down between them (*fig. 25*). Meanwhile the nuclear membrane is formed and the chromatic elements pass over into the reticulum characteristic of the resting state.

Finally, the new cell plates unite with the folded walls between the lobes and the separation of the spores is complete (*fig. 26*). The contiguous walls split apart and the spores become free. They next increase in size, becoming almost spherical, and the wall thickens and is finally marked with delicate points (*fig. 27*).

The spores do not germinate in the capsule as do the spores of Pellia. Soon after being shed, they increase greatly in size, stretching the wall, as is clearly shown by the separation of the points upon its surface. After the cell has attained a size several times that of the

original spore, the first mitosis of the gametophyte generation takes place.

I have not been able to contribute much to a knowledge of the behavior of the nucleolus. It stains like the chromosomes most of the time, and when the latter are differentiated it becomes difficult to identify the nucleolus with certainty. During synapsis the nucleolus is a very large and conspicuous body (*fig. 1*). It is not so large during the later spirem stages, but still quite prominent (*figs. 2* and *3*). At the time the spirem is ready to segment, the nucleolus shows a slight difference in staining reaction from the chromosomes. With the saffranin and gentian violet combination it takes slightly more gentian violet, and with the iron-alum haematoxylin it stains less intensely than the chromosomes. At this time it shows signs of fragmentation (*figs. 6* and *9*).

Various theories regarding the constitution of the nucleolus have been advanced: one that it is achromatic and contributes to the formation of the spindle; another that it is chromatic and contributes to the formation of the chromosomes. WAGER (**18**) in a recent paper attributes to it important functions in the organization of the chromosomes and in the transmission of the hereditary substance. Its staining reactions would seem to ally it more closely with the chromatic elements of the cell. If the nucleolus plays a part in the formation of the achromatic spindle in the first division of Pallavicinia, it certainly does not in the second, since there is no reconstruction of the nucleus and the nucleolus is not reformed. Upon the whole the evidence, though by no means conclusive, indicates that the nucleolus in Pallavicinia may be regarded as contributing to the chromatin.

FARMER (**6**) states that there are four chromosomes in *Pallavicinia decipiens*. In *P. Lyellii* I find eight as the reduced number in the spore. The count is very easily made when a polar view is obtained, and the compact form of the chromosomes makes the task an easy one. The chromosomes are in most favorable position for counting when viewed from the poles during metaphase and early telophase, as the figures clearly show (*figs. 8, 9, 21, 23*).

In *fig. 23* it will be observed that there are nine chromosomes in one group. It is possible that the sister group would show only seven. In the same figure, upon the conspicuous spindle which is

cut longitudinally it is uncertain to which group the chromosome lying near the middle belongs. *Fig. 18* shows that the chromosomes do not always pass simultaneously to the poles, and it is possible that the distribution is not always equal. I have frequently been able to count only seven chromosomes in a group, but such evidence is uncertain, since there is always the possibility that one has been removed by the razor in making the section. In case the number exceeds eight the difficulties are fully as great, since there is always the possibility of a tetrad being broken apart. It is true that in such an example the size of the bodies is some check, but still there is great uncertainty. Also the nucleolus, which as has been stated stains as the chromosomes, is to be reckoned with, if the count is made at a stage when that body is present. However, I believe that while the number of the chromosomes is normally eight, occasionally a variation from this number will be met, due no doubt to an unequal distribution during division.

The number of chromosomes in the sporophyte is undoubtedly sixteen, though I have not made an actual count. *Figs. 29* and *30* represent the two parts into which a single cell of the seta has been cut. It will be observed that the spirem is just segmenting into the elongated chromosomes; two nucleoli are still visible (*fig. 29*). The count cannot be made with absolute certainty, but the number is approximately sixteen. *Fig. 31* shows one section of an early telophase from a cell of the seta. There are seven and eight chromosomes at the respective poles. The other section of the same cell shows about the same number of chromosomes in each group, but the masses are too confused to admit of an accurate count. I have observed figures in dividing spermatogenous cells, and here also the number of chromosomes is without doubt sixteen.

It seems desirable to point out that my final conclusions agree in all essentials with my preliminary paper of 1903, and are in conflict with FARMER'S views in the fundamental feature of his account of Pallavicinia—the simultaneous distribution of the chromatin to the four daughter nuclei through a quadripolar spindle. It is perfectly clear from my studies that the chromosomes in *Pallavicinia Lyellii* are distributed by two successive mitoses, each with well-defined bipolar spindles, and that the chromosomes are organized

as tetrads just before the first mitosis. The achromatic structure which corresponds to FARMER'S quadripolar spindle appears during the prophase of the first nuclear division, and is followed by clearly defined bipolar spindles of the two successive mitoses with no evidence of accompanying centrosomes. The events of sporogenesis in *Pallavicinia Lyellii* present then no fundamental differences from those of other liverworts and higher plants, the chief peculiarity being the rapidity with which the second mitosis follows the first.

SUMMARY.

1. The resting nucleus is spherical in shape and centrally situated in the spore mother cell. The spore mother cell is deeply four-lobed at an early period in its history.

2. During synapsis the nucleus, containing a large and conspicuous nucleolus and a contracted chromatic thread, enlarges and becomes irregularly lobed.

3. There is a distinct spirem stage in which a clear cut linin thread bears deeply staining chromatin granules. The thread shortens and thickens and at the same time the granules become larger and less numerous.

4. The first evidence of a double spirem is observed just previous to the segmentation of the thread.

5. The spirem segments into eight tetrads, which may be in the form of rings, Xs, Ys, Ts, or irregular masses.

6. While these changes are taking place within the nucleus, the membrane becomes strongly lobed. Frequently, though not always, the form of the nucleus is tetrahedral, the angles projecting into the respective lobes of the spore mother cell.

7. There is no direct evidence of centrosomes or centrospheres and the indirect evidence is against their presence.

8. The lobing of the nucleus is due to amoeboid motion in response to nutritive stimuli.

9. The achromatic spindle originates in kinoplasmic caps to which the nuclear membrane contributes material.

10. The distribution of the chromatin is effected through bipolar spindles in two successive mitoses.

11. There is no resting stage between the first and second mitoses.

12. The two bipolar spindles of the second mitosis are strongly developed and stand at right angles to each other.

13. After the second mitosis, cell plates are formed and the nuclei pass into a condition of rest in the usual manner.

14. The spores do not germinate in the capsule before its rupture, as do those of Pellia.

15. The nucleolus is more closely allied to the chromatic than to the achromatic material of the nucleus.

16. The number of chromosomes for the gametophyte is eight and for the sporophyte sixteen.

This investigation was conducted under the direction of Professor BRADLEY M. DAVIS, to whom I desire to acknowledge my indebtedness for valuable criticism and suggestion; as well as to Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN.

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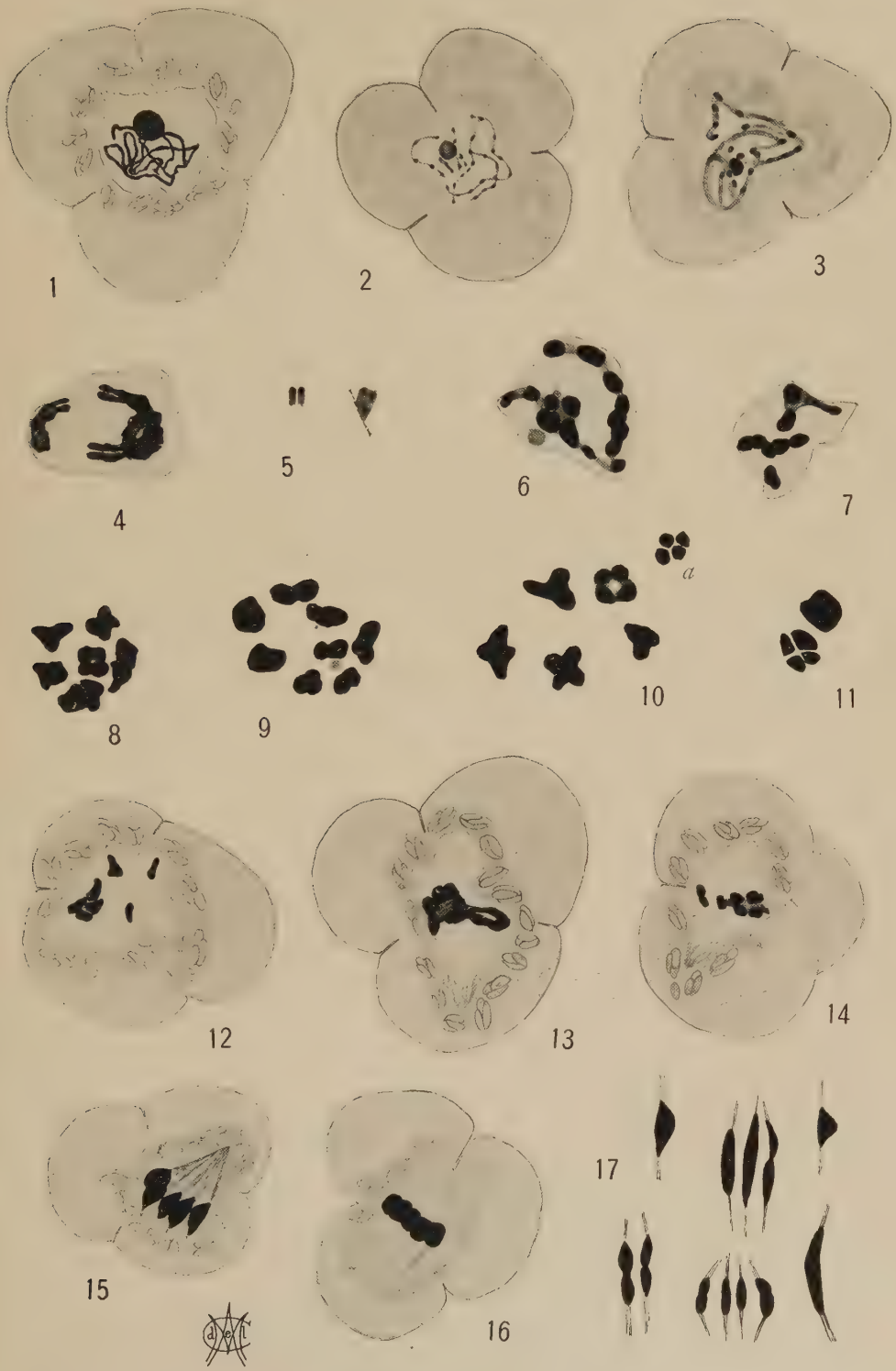
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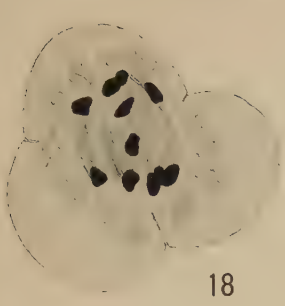
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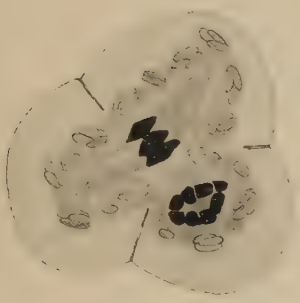
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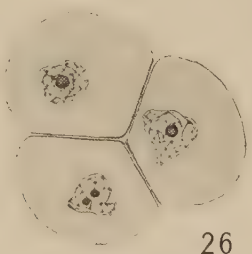
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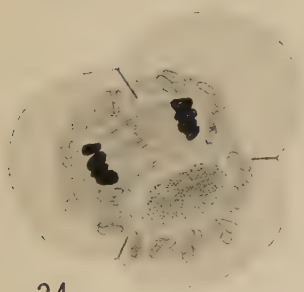
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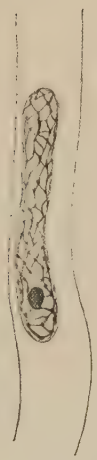
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EXPLANATION OF PLATES III AND IV

All figures except *fig. 28* were made with a Zeiss 2^{mm} apochromatic objective and a no. 12 ocular. *Fig. 28* was made with the same objective, but with no. 8 ocular. A Bausch and Lomb camera lucida was used for all drawings. In *fig. 2* all the plastids of the cell are shown; in other cases only those immediately surrounding the nucleus.

FIG. 1. Enlarged nucleus of spore mother cell in early stage of preparation for division; the nucleolus is conspicuous and the appearance and arrangement of the chromatin indicate the condition of synapsis.

FIG. 2. Spirem condition, showing linin thread loosely wound with deeply staining chromatic droplets at intervals.

FIG. 3. Thicker and shorter spirem; chromatic droplets fewer and larger; lobes of nucleus distinctly rounded.

FIG. 4. Spirem further shortened; chromatic droplets crowded together; the thread appears double.

FIG. 5. Ends of spirem thread, showing that it is double.

FIG. 6. Aggregation of chromatic droplets just previous to segmentation of chromosomes; probably time of tetrad formation; the nucleolus seems to be fragmenting.

FIG. 7. Later stage than *fig. 6.*

FIG. 8. Equatorial plate stage, showing group of eight tetrads.

FIG. 9. Equatorial plate stage, later than *fig. 8;* tetrads not so clearly defined; nucleolus fragmenting.

FIG. 10. A group of selected tetrads, showing rings, crosses, Ys, and Ts; *a*, tetrad resolved into its elements.

FIG. 11. Neighboring tetrads of an equatorial plate; in one the fourfold character is clear, while in the other it is obscured.

FIG. 12. Prophase of first division; nucleus many-lobed; fibers over the largest lobe and at other places on the surface of the nucleus.

FIG. 13. Spindle organizing for first division; spindle fibers prominent on one end, approaching bipolar condition.

FIG. 14. Bipolar spindle of first division; nuclear membrane resolving into spindle fibers.

FIG. 15. Oblique side view, metaphase of first division; end of spindle pointed.

FIG. 16. Metaphase of first division, showing one very flat and one forked pole.

FIG. 17. Dividing chromosomes.

FIG. 18. Anaphase of first division, showing chromosomes scattered.

FIG. 19. Telophase of first division, showing grouping of chromosomes in rings at the poles.

FIG. 20. Beginning of reconstruction of daughter nuclei at completion of first division; the chromosomes do not lose their identity and no nuclear membrane is formed.

FIG. 21. Metaphase of second division, showing side view of one spindle and polar view of the other; in the side view the poles are seen to be pointed and in the polar view eight chromosomes appear.

FIG. 22. Anaphase of second division, showing blunt poles.

FIG. 23. Telophase of second division, showing nine chromosomes in the polar view of one of the spindles.

FIG. 24. Telophase of second division, showing beginning of cell plate in one spindle and transverse section of fibers in the other.

FIG. 25. Formation of cell plate.

FIG. 26. Completed spores with resting nuclei and separating walls.

FIG. 27. A single spore which has increased in size and has attained its thickened and roughened wall.

FIG. 28. Resting nucleus of elater.

FIG. 29. Segmenting spirem of cell from seta of sporophyte, showing nine chromosomes.

FIG. 30. Remainder of the same cell, showing seven additional chromosomes, making sixteen in all; chromosomes differ in shape from those of the dividing spore mother cell.

FIG. 31. Early telophase of cell from seta; only half of the cell is shown; there are eight chromosomes at one end and seven at the other; the neighboring section makes it evident that the total number is sixteen at each end.